REMARKS

Applicants have substituted into the present specification a new paper copy Sequence Listing section according to 37 C.F.R. §1.821(c). Furthermore, attached hereto is a 3 1/2" disk containing the "Sequence Listing" in computer readable form in accordance with 37 C.F.R. §1.821(e).

Applicants have amended the specification to insert SEQ ID Nos, as supported in the present specification.

The following statement is provided to meet the requirements of 37 C.F.R. §1.825(a) and 1.825(b).

I hereby state, in accordance with 37 C.F.R. §1.825(a), that the amendments included in the substitute sheets of the sequence listing are believed to be supported in the application as filed and that the substitute sheets of the sequence listing are not believed to include new matter.

I hereby further state, in accordance with 37 C.F.R. §1.825(b), that the attached copy of the computer readable form is the same as the attached substitute paper copy of the sequence listing.

Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current

amendment. The attached page is captioned "Version with markings to show changes made".

Applicants submit that the present application contains patentable subject matter and therefore urge the examiner to pass the case to issuance.

If the examiner has any questions or comments concerning the above described application, the examiner is urged to contact the undersigned at the phone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at page 13, line 13, has been replaced with the following rewritten paragraph:

"Colony primer" as used herein refers to an entity which comprises an oligonucleotide sequence which is capable of hybridizing to a complementary sequence and initiate a specific polymerase reaction. The sequence comprising the colony primer is chosen such that it has maximal hybridising activity with its complementary sequence and very low non-specific hybridising activity to any other sequence. The sequence to be used as a colony primer can include any sequence, but preferably includes 5'-AGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO:1) or 5'-CACCAACCCAAACCAACCCAAACC (SEQ ID NO:2). The colony primer can be 5 to 100 bases in length, but preferably 15 to 25 bases in length. Naturally occurring or non-naturally occurring nucleotides may be present in the primer. One or two different colony primers may be used to generate nucleic acid colonies in the methods of the present invention. The colony primers for use in the present invention may also include degenerate primer sequences.

The paragraph beginning at the bottom of page 44, line 28, has been replaced with the following rewritten paragraph:

Figure 8: shows hybridization of probes to oligonucleotides attached to Nucleolink, before and after PCR cycling. The figure shows R58 hybridization to CP2 (5'-

(phosphate)- TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 closed circles, CP8 (5'(amino-hexamethylene)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 closed triangles, CP9 (5'(hydroxyl)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 diamonds, CP10 (5'(dimethoxytrityl)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 open circles and CP11 (5'(biotin)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 open triangles.

The paragraph beginning at page 45, line 16, has been replaced with the following rewritten paragraph:

The properties of the colony primers have been chosen based on a selection for oligonucleotide primers that show little non-specific nucleotide incorporation in the presence of heat-stable DNA polymerases. The colony primers, CP (5'-p CACCAACCCAAACCAACCCAAACC) SEQ ID NO:2 and CP (5'-p AGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:1 have been selected due to their low incorporation of radiolabeled [^{32}P-dCTP] in the presence of a stable DNA polymerase (AmpliTaq, Perkin Elmer, Foster City, CA) in the standard buffer and under thermocycling conditions (94 C for 30 seconds, 65 C for 1 minute, 72 C for 2 minutes, 50 cycles).

The Table 1, beginning on page 48, has been replace with the following new Table 1:

TABLE 1

List of oligonucleotides used for templates preparation and colonies generation:

Name	DNA sequence	Coordinates (orientation)	Oligonucleotide Modification	Use
TP1	GAGGCCAGAACAGTTCAAGG (SEQ ID NO:3)	9810 (R)		Template 3.2 Kb
TP2	CCTGTGACAAGACGACTGAA (SEQ ID NO:4)	6550 (F)		Template 3.2 Kb
CP1	TTTTTTTTTTCACCAACCCAAAC CAACCCAAACC (SEQ ID NO:5)	None	5'P	Generate colonies
CP2	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:6)	None	5'P	Generate colonies
CP3	TTTTTTTTTTCACCAACCCAAAC CAACCCAAACC (SEQ ID NO:7)	None	5'SH	Generate colonies
CP4	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:8)	None	5'SH	Generate colonies
CP5	AGAAGGAGAAGGAAAGGGAAAGG GTTTTTTTTTTTTTTTNN (SEQ ID NO:9)	None	5'P	Generate colonies
CP6	AGAAGGAGAAGGAAAGGGAAAGG GGG (SEQ ID NO:10)	None	5'P	Generate colonies
CP7	TTTTTTTTTTCACCAACCCAAAC CAACCCAAACC (SEQ ID NO:5)	None	5'(NH_2)	Generate colonies
CP8	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:6)	None	5'(NH_2)	Generate colonies
CP9	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:6)	None	5'(OH)	Control oligo
CP10	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:6)	None	5'(DMT)	Control oligo
CP11	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGG (SEQ ID NO:6)	None	5'(biotin)	Control oligo
TPA1	AGAAGGAGAAGGAAAGGGAAAGG GCCTGTGACAAGACGACTGAA (SEQ ID NO:12)	6550 (F)	5'P	Template A
TPA2	TTTTTTTTTTAGAAGGAGAAGGA AAGGGAAAGGGGCGGCCGCTGAG GCCAGTGGAAGTCAGA (SEQ ID NO:13)	7403 (R)	5'P	Template A
TPB3	TTTTTTTTTTCACCAACCCAAAC CAACCCAAACCGAGCTCAGGCTG AGGCAGGAGAATTG (SEQ ID NO:14)	9049 (F)	None	Template B'
TPB1	AGAAGGAGAAGGAAAGGGAAAGG GGAGCTGAGGAGGAAGAGAGG (SEQ ID NO:15)	9265 (F)	None	Template B
TPB2	AGAAGGAGAAGGAAAGGGAAAGG GGCGGCCGCTCGCCTGGTTCTGG AAGACA (SEQ ID NO:16)	8411 (R)	5'P	Template B
TPB4	AGAAGGAGAAGGAAAGGGAAAGG GGCGGCCGCTCGCCTGGTTCTGG AAGACA (SEQ ID NO:11)	9265 (R)	5'SH	Template B'

Coordinate from HUMOXRAGE gene Accession number D28769
(R) means "reverse" and (F) means "forward"

The paragraph beginning at the bottom of page 49, line 15, has been replaced with the following rewritten paragraph:

A 3.2 Kb DNA fragment was taken as a model system to demonstrate the feasibility of colony generation from random primer PCR amplification. This strategy can be applied to sequencing of DNA fragments of approximately 100 Kb in length and, by combination of fragments to whole genomes. A fragment of DNA of 3.2 Kb was generated by PCR from human genomic DNA using PCR primers; TP1 5'-pGAGGCCAGAACAGTTCAAGG (SEQ ID NO:3)and TP2 5'-pCCTGTGACAAGACGACTGAA SEQ ID NO:4 as described in example 1. The 3.2 Kb fragment was cut in smaller fragments by a combination of restriction enzymes (EcoR1 and HhaI yielding 4 fragments of roughly 800 bp). The cut or uncut fragment DNAs were then mixed with the degenerate primer, p252 (5'-P TTTTTTTTTTISISISISISIS, SEQ ID NO:17 where I stands for inosine (which pairs with A, T and C) and S stands for G or C) and covalently coupled to the Nucleolink wells (Nunc, Denmark). The tubes were then subjected to random solid phase PCR amplification and visualized by hybridisation with labeled DNA probes, as will be described in Example 2a.

The paragraph beginning at page 50, line 17, has been replaced with the following rewritten paragraph:

A colony primer (CP2, 5'-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6,

phosphorylated at its 5' terminus (Microsynth GmBH, Switzerland), was attached onto Nucleolink plastic microtitre wells (Nunc, Denmark) in the presence of varying doses of Template A (prepared as described in example 1). 8 wells were set up in duplicate with seven 1/10 dilutions of template with CP2, starting with the highest concentration of 1 nM.

The paragraph beginning at page 51, line 24, has been replaced with the following rewritten paragraph:

Colonies Visualization

Probe: The probe was a DNA fragment of 1405 base pairs comprising the sequence of the template at their 3' end (nucleotide positions 8405 to 9259). The DNA probe was synthesized by PCR using two primers: p47 (5'-GGCTAGGAGCTGAGGAGGAA) SEQ ID NO:20, amplifying from base 8405, and TP2, biotinylated at 5' end, amplifying from base 9876 of the antisense strand.

The paragraph beginning at page 54, line 21, has been replaced with the following rewritten paragraph:

A colony primer (CP2: 5'pTTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:8, phosphorylated at its 5' termini (Microsynth GmbH, Switzerland), was grafted onto Nucleolink plastic microtitre wells (Nunc, Denmark) in the presence of varying doses of the two templates A and B (prepared as described in example 1). Series of 8 wells were set up in triplicate with seven 1/10 dilutions of both templates starting with the highest

concentration of 1 nM. Template dilutions are set up in opposite directions such that the highest concentration of one template coincides with the lowest of the other.

The paragraph beginning at page 67, line 5, has been replaced with the following rewritten paragraph:

Colony primers CP1 (5'-pTTTTTTTTTTCACCAACCCAAACCAACCCAAACC) SEQ ID NO:7 and CP2 (5'-pTTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 which are 5'phosphorylated (Microsynth GmbH, Switzerland) and DNA template B (prepared as described in example 1) were 5' covalently attached onto 5 mm diameter glass slides (Verrerie de Carouge, Switzerland) to a final concentrations of 1 µM and 10 nM respectively, as follows: 2 nmoles of each primer were added to 0.2 nmoles of template in 1 ml of solution A (41 µl of Methylimidazole (Sigma, #M-8878) in 50 ml H_2O, pH adjusted to 7 with HCl) and then mixed 1:1 with solution D (0.2 mM EDC in 10 ml of solution A). On both glass slides sides, 3.5 µl of the mixture were loaded, and incubated over night at room temperature. The glass slides were then briefly rinsed with 5xSSC buffer and placed at 100 C in 10mM Tris buffer pH 8.0 for 2x5'.

The paragraph beginning at page 67, line 27, has been replaced with the following rewritten paragraph:

Glass slides were then individually placed onto a Microamp™ reaction tube (Perkin Elmer) containing 170 µl of PCR mix, and DNA colonies were then generated using Taq

polymerase (AmpliTaq, PE-Applied Biosystems Inc., Foster City CA) with 50 cycles (94C/60", 60C/3', 72C/6') in a MTC 200 thermo-cycler (MJ Research, Watertown, MA). Each slide was digested twice using 1.3 units of Pvu II (Stratagene) in NEB 2 buffer (New England Biolabs) for 45 minutes at 37 C. After digestion, the tubes were placed at 100 C in 10mM Tris buffer pH 8.0 for 2x5', then blocked with filtered (Millex GV4, Millipore) 1 mg/ml BSA in 2xSSC buffer for 30' at room temperature and rinsed first in 2xSSC 0.1% SDS buffer then in 5xSSC buffer. Each slide was incubated over night at room temperature with a 5xSSC/0.1% Tween 20 buffer containing 1 µM of the sequencing primer p181 (CGACAGCCGGAAGGAAGAGGGAGC) SEQ ID NO:18 overnight at room temperature. Controls without primer were kept in 5xSSC 0.1% Tween 20 buffer. Glass slides were washed 2 times in 5xSSC 0.1% SDS at 37C for 5' and rinsed in 5xSSC. Primer p181 can hybridize to template B' and the sequence following p181 is CAGCT.... In order to facilitate focusing, green fluorescent beads have been adsorbed to the bottom of the well by incubating each well with 20 µl of a 1/2000 dilution of 200 nm yellow/green fluorescent, streptavidin coated FluoSpheres(R) (Molecular Probes, Eugene, OR) in 5X SSC for 20" at room temperature.

The paragraph beginning at page 71, line 19, has been replaced with the following rewritten paragraph:

cDNA synthesis - Synthetic mRNA was mixed with mouse liver poly A+ mRNA at different molar ratios (1:1, 1:10, 1:100) and cDNA synthesis on the mixture of synthetic and

mouse liver mRNA was performed using the "SMART PCR cDNA synthesis kit" (Clontech, Palo Alto CA) with some minor modifications. In a cDNA reaction, approximately 1 µg of the mRNA mixture was mixed with the -primer CP5, having at the 5' -end the sequence of CP, (5'p-AGAAGGAGAAGGAAAGGGAAAGGGTTTTTTTTTTTTTTTTNN) SEQ ID NO:9. This primer has been used to make the 1st strand cDNA synthesis. For the 2nd strand synthesis, the "SMART" technique has been used. The basis of the SMART synthesis is the property of the Moloney murine viral reverse transcriptase to add three to five deoxycytosine residues at the 3'-termini of first strand cDNA, when the mRNA contains a 5'-methylguanosine-cap (SMART user manual, Clontech, Palo Alto CA). A CP6 primer, which contains the sequence of CP plus AAAGGGGG (SEQ ID NO:21) at the 3' end, (5'p-AGAAGGAGAAGGAAAGGGAAAGGGGG) SEQ ID NO:10 has been used for the 2nd strand cDNA synthesis. Buffer and SUPERSCRIPT™ II RNase H- reverse transcriptase from Moloney murine leukemia virus (Life Technologies, Ltd.) were used as described in the instructions and the reaction was carried out at 42 C for 1 hr. The cDNA was assayed by PCR using the primer p251, which contains a fragment of the CP sequence, (5'-GAGAAGGAAAGGGAAAGG) SEQ ID NO:19 with Taq DNA polymerase (Platinum Taq, Life Technologies, Ltd.).

The paragraph beginning at page 72, line 10, has been replaced with the following rewritten paragraph:

Preparation of DNA colonies - The 5'p-cDNA was mixed

with different concentrations of the solid phase colony primer, CP2 (5'p-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 and chemically bound to Nucleolink PCR tubes (NUNC) following manufacturer instructions. DNA colonies were then generated using Taq polymerase (AmpliTaq Gold, PE-Applied Biosystems Inc., Foster City CA) with 30 cycles (94C/30", 65C/1', 72C/ 1.5') in a MTC 200 thermo-cycler (MJ Research, Watertown, MA).

The paragraph beginning at page 74, line 16, has been replaced with the following rewritten paragraph:

Oligonucleotide primers were attached onto Nucleolink plastic microtitre wells (Nunc, Denmark) in order to determine optimal coupling times and chemistries. Oligonucleotides; CP2 (5'-(phosphate)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6, CP8(5'-(amino-hexamethylene)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6, CP9 (5'(hydroxyl)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6, CP10 (5'-(dimethoxytrityl)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6 and CP11 (5'(biotin)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) SEQ ID NO:6, (Microsynth GmbH, Switzerland), were attached to Nucleolink microtitre wells as follows (8 wells each); to each well 20 µl of a solution containing 0.1 µM oligonucleotide, 10mM 1-methyl-imidazole (pH 7.0) (Sigma Chemicals) and 10mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (pH 7.0) (Sigma Chemicals) in 10mM 1-methyl-imidazole. The wells were then sealed and incubated 50°C for varying amounts of time. The

coupling reaction was terminated at specific times by rinsing twice with 200 µl of RS (0.4 N NaOH, 0.25% Tween 20) and twice with 200 µl TNT (100mM TrisHCl pH 7.5, 150 mM NaCl, 0.1% Tween 20). Tubes were dried at 50°C for 30' and were stored in a sealed plastic bag at 4°C.

The paragraph beginning at page 75, line 6, has been replaced with the following rewritten paragraph:

Stability was tested under colony growing conditions by adding a PCR mix (20 µl of four dNTPs (0.2 mM), 0.1% BSA, 0.1% Tween 20, 8% DMSO (dimethylsulfoxide, Fluka, Switzerland), IX PCR buffer). The wells were then placed in the thermocycler and for 33 repetitions under the following conditions: 94°C for 45 seconds, 60°C for 4 minutes, 72°C for 4 minutes. After completion of this program, the wells were rinsed with 5xSSC, 0.1% Tween 20 and kept at 8°C until further use. Prior to hybridization wells are filled with 50 µl 5xSSC, 0.1% Tween 20 heated at 94°C for 5 minutes and stored at RT.

Probe: Oligonucleotide probes, R57 (5'(phosphate)-GTTTGGGTTGGTTTGGGTTGGTG (SEQ ID NO:22), control probe) and R58 (5'-(phosphate)-CCCTTTCCCTTTCCTTCTCCTTCT (SEQ ID NO:23), which is complementary to CP2, CP8, CP9, CP10 and CP11) were enzymatically labeled at their 5' end terminus with [$-^{32}$P]dATP (Amersham, UK) using the bacteriophage T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Excess ^{32}P dATP was removed with a Chroma Spin column TE-10 (Clontech, Palo Alto CA). Radiolabeled oligonucleotides (0.5 µM in 5xSSC, 0.1% Tween 20) were then hybridized to the oligonucleotide

derivatized Nucleolink wells at 37°C for two hours. The wells were washed 4 times with 5xSSC, 0.1% Tween 20 at room temperature, followed by a wash with 0.5xSSC, 0.1% Tween 20 for 15' at 37°C. Wells were then assayed for bound probe by scintillation counting.